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Technical Information Manager: Angela Moore (513) 569-7341

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ABSTRCT/ORAL

Fayer, R., Trout, J., Sulaiman, I.M., Lal, A.A., Xiao, L., and Schaefer, F.W. The first report of Enterocytozoon bieneusi parasites in wildlife: Implications for public health and transmission to livestock. Presented at: 10th International Congress of Parasitology, Vancouver, BC, August 4-9, 2002. — 8/4/2002

Contact: Frank W. Schaefer

Abstract:

Graczyk, T., Bosco-Nizeyi, J., Innocent, R. B., DaSilva, A. J., Pieniazek, N. J., Lindquist, H.D.A., and Cranfield, M. R. A single genotype of Encephalitozoon intestiinalis infects free-ranging gorillas and people sharing their habitats, Uganda. Presented at: International Conference on Emerging Infectious Diseases, Atlanta, GA, March 24-27, 2002. — 3/24/2002

Contact: H. d. alan Lindquist

Abstract: For conservation purposes and due to ecotourism free-ranging gorillas of Uganda have been habituated to humans, and molecular epidemiology evidence indicates that this habituation might have enhanced transmission of anthropozoonotic pathogens. Microsporidian spores have been detected by modifid trichome and calcofluor stains in fecal samples of 3 gorillas and 2 people sharing gorilla habitats. All spore isolates have been identified by polymerase chain reaction (PCR) and fluorescent in situ hybridization (FISH) to be Encephalitozoon intestinalis. Subsequent sequencing of the PCR product demonstrated that all isolates represented a single genotype of E. intestinalis. A single genotype in two genetically distant but geographically united host groups indicats anthropozoonotic transmission of E. intestinalis. It is unlikely that infections with genetically identical pathogens were acquired independently, and it is much more likely that one of these two host groups initiated infection of the other group.

Marshall, M.M., Hoffman, B., Moffett, J., Jost, H., Polchert, D., Ware, M.W., Stubaum, G., and Battigelli, D. Identification and characterization of infectious and non-infectious sub-populations of Encephalitizoon intestinalis spores purified from in vitro cell culture. Presented at: International Symposium on Waterborne Pathogens, Lisbon, Portugal, September 22-25, 2002. — 9/22/2002

Contact: Michael W. Ware

Abstract: Background: Encephalitizoon intestinalis spores were propagated in rabbit kidney (RK-13) cells and were purified using density gradient (Percoll [registered trademark]) centrifugation. Purified spores were enumeraged and aliquotted using flow cytometry with cell sorting for use in dose response assays. Flow cytometry analyses suggested a heterogeneous distribution of spore size, with two distinct subpopulations. This observation was confirmed in independent laboratories that enumerated separate lots of purified E. intestinalis spores.Project Description and Results: Viability dye staining of sorted spores showed that the smaller subpopulation was propidium iodide (PI) - positive and 4',6-diamidino-2-phyenylindole diacetate (DAPI) - positive while the larger subpopulation was PI- and only stained DAPI+ following pretreatment with ethanol. Approximately 18-20% of any batch of purified E. intestinalis spores were of the size range of the larger subpopulation. This observation was confirmed by evaluating 12 batches of E. intestinalis spores produced and purified over a 12 month period. Spores of each subpopulation were subsequently infected into RK-13 cells using an optimized in vitro cell culture coverslip assay. The larger spores were infective in the cell culture assay, whereas the smaller spores were noninfectious in RK-13 cells. To confirm identity of the subpopulations, spore DNA was subjected to polymerase chain reactoin (PcR) assay using primers directed against the 16S rRNA or B-tubulin genes. PCR products were sequenced and compared to those of the E. intestinalis sequences for 16S rRNA or B-tubulin genes deposited in GenBank. Sequence data indicated that both the large and small spore populations possessed E. intestinalisB-tubulin and 16S rRNA genes, and were not derived from contaminants.Significance: These data highlight the fact that not all E. intestinalis spores purified form cell culture are infective or viable. Furthermore, the large number (90-82%) of non-viable/non-infectious spores must be taken in to account when designing microsporidia spore disinfection studies. The number of non-viable/non-infectious spores in any given dose could potentially result in significant over-estimation (1.0 log10 or greater) of viability reduction. Researchers should be aware that there is inherent variability in cell culture spore production. Furthermore, the viability of a batch of E. intestinalis spores purified from RK-13 cells may be as low as 20% of the total number of spores.

Piche, R., Chen, R., Hoang, L., Cross, J.H., Lindquist, H.D.A., Fyfe, M.W., Champange, S., Isacc-Renton, J.L., and Ong, C.S.L. Molecular epidemiological investigation of 2 cyclosporiasis outbreaks in Vancouver, British Columbia. Presented at: Joint meeting of the International Congress of Parasitology & Annual Meeting of the American Society of Parasitologists, Vancouver, British Columbia, Canada, August 4-10, 2002. 8/4/2002

Contact: H. d. alan Lindquist

Abstract: Introduction: Cyclospora cayentanensis is a waterborne apicocomplexan protozoan that has been recognized as an emerging parasite. This parasite is the cause of severe diarrhea, which can only be treated with sulfa drugs. Cyclospora caytentanensis is endemic in some parts of the world such as Guatemala and Nepal. In North America, local outbreaks are usually foodborne acquired from the consumption of imported fruits and vegetables. Outbreaks of Cyclospora cayentanensis occurred in BC in 1999 and 2001. The internal transcribed sequence 1 (ITS1) region located between the 18S and the 5.8S rRNA genes is thought to have potential for use in genotyping the protozoan. As part of the outbreak investigation the ITS1 region was sequenced to look for any variation in genotype between different specimens. Methods: Stool samples were collected from cases in the Cyclospora cayentanensis outbreaks as well as sporadic cases in BC and Nepal. Although the cause of the 1999 outbreak has not been identified, the 2001 outbreak was linked epidemiologically to the consumption of Thai basil. Cycle sequencing of the 18S rRNA and ITS1 genes was performed after PCR amplification and spin-column purification. Sequences were assembled and aligned using Clustal X.Results: Eighteen stool specimens were analyzed, 6 of which were collected from the 1999 outbreak, 6 from the 2001 outbreak, 5 from BC sporadic and 1 from Nepal sporadic cases. 18S rDNA sequences of five samples collected in the 1999 outbreak, and four samples collected in 2001 were obtained. These sequences showed almost compete homology. The only discrepancy was two single base pair variations in one sample. The ITS1 sequence was obtained for four of the 1999 samples and three of the 2001 samples. The sequences all showed a large degree of homology. The greatest variation was in a 2001 sample obtained from a sporadic case in BC around the time of the 2001 outbreak. This sequence contained insertions and single base pair variation.

Fayer, R., Santin, M., Sulaiman, I.M., Trout, J., Xunde, L., Schaefer, F.W., Xiao, L., and Lal, A.A. Animal reservoirs, vectors, and transmission of microsporidia. Presented at: American Soecity of Tropical Medicine and Hygiene 51st Annnual Meeting, Denver, CO, November 10-14, 2002. 11/10/2002

Contact: Frank W. Schaefer

Abstract: Fourteen species of microsporidia have been identified as opportunistic or emerging pathogens of humans. Several genotypes of Enterocytozoon bieneusi, the most frequently diagnosed species in humans, have been identified in Europe in farm and companion animals including pigs, cattle, cats, dogs, a llama, and chickens. Of 88 animals examined from herds in Germany, E. bieneusi has been identified in 8 calves. Molecular techniques were developed to determine the prevalence of E. bieneusi in calves on farms in North America. Of 300 preweaned calves from 5 states, 46 calves were PCR positive for microsporidia and 5 of these were positive for E. bieneusi. Wildlife that inhabit wetlands adjacent to farming areas were also examined. Feces from over 400 beavers, foxes, muskrat, otters, and raccoons, trapped in eastern Maryland, were examined for microsporidia using a 2-step nested PCR protocol for E. bieneusi. Fifty-nine samples were sequenced and multiple alignments of these sequences identified 15 genotypes of E. bieneusi of which 13 have not been reported before. Most genotypes were found in multiple species of wildlife. Some isolates from muskrats and raccoons formed two distinct groups, while the rest belong to a major group with all the previously described E. bieneusi genotypes from human and non-human sources. These findings indicate that livestock and wildlife can be potential sources of human-pathogenic E. bieneusi.

Presented *Published*

Seys, S.A., Mainzer, H.M., Heryford, A.G., Anderson, A.D., Monroe, S.S., Fout, G.S., Sarisky, J.P., and Musgrave, K.J. Coordinating systems-based environmental public health practice with epidemiology and laboratory analysis: a waterborne outbreak of Norwalk-like virus in the Big Horn Mountains of Wyoming. Presented at: American Public Health Association Annual Meeting, Philadelphia, PA, November 9-13, 2002. 11/9/2002

Contact: G. shay Fout

Abstract: Background: In February 2001, the Wyoming Department of Health received reports of cases of gastroenteritis among persons who had been snowmobiling in the Big Horn Mountains. Laboratory testing suggested that exposure to a Norwalk-like virus was responsible for the illness.Methods: Environmental health specialists and epidemiologists from several state and federal agencies coordinated an investigation of environmental risk factors and system-based failures. The environmental assessment of three area lodges included food service operations, water supply systems, and sewage disposal. A retrospective cohort study was conducted among 82 guests to identify risk factors associated with illness. Stool and water samples were collected for laboratory analysis.Results : Statistical analysis suggested that illness was associated with water consumed at one of the three area lodges (RR=3.3, 95% C.I.=(1.4,7.7)). Reverse transcriptase-polymerase chain reaction (RT-PCR) testing on 13 stool samples yielded 8 positives for Norwalk-like virus (NLV) genogroup II, with 3 distinct sequence types detected. Fecal contaminaiton of one of three operating wells was also found and one of the samples tested positive for NLV genogroup II. The environmental assessment of the property revealed that an inadequately installed sewage system was delivering effluent into shallow soil with poor filtering capacity.Conclusion: This outbreak illustrates waterborne transmission of viral gastroenteritis and the advantages of coordinating a systems-based model of enviromental asssessment with traditional epidemiologic and laboratory practices. Preventing future system deficiencies depends on understanding the relevant protective components of facility operations including the surrounding physical environment, food service, water supply, and sewage systems.

Schaefer, III, F.W. Utility of surrogates for measuring Cryptosporidium oocysts infectivity. Presented at: Drinking Water Inspectorate Research Conference, Tadley Court, UK, June 10-11, 2002. 6/10/2002

Contact: Frank W. Schaefer

Abstract: The water industry must assess whether Cryptosporidium oocysts detected in source and finished water are viable and/or infectious. Initial approaches measuring the infectious nature of C. parvum oocysts have focused on in vitro excystation and in vitro vital dye staining. Recently there has been increased interest in animal models and a Cryptosporidium cell culture assay. Studies comparing in vitro assays to over estimate the infectious nature of oocysts exposed to disinfectants. While human volunteer studies have been conducted to determine the infectious dose of various Cryptosporidium isolates, human volunteers have never been used for disinfection studies. Besides being extremely expensive, it is difficult to get human subjects approval to do such infectivity studies. Animal models have been used with some success, but they are only able to evaluate C. parvum genotype 2 isolated from bovids and humans and not C. parvum genotype 1 isolated only from humans. In addition, results from both human volunteers and animal models are extremely variable, as they are dependent on the strain and age of the host, how long it has been since the oocysts were shed, the strain and isolate of C. parvum, the methods used to enumerate the oocysts, the procedures used to inoculate the oocysts, and the inter- and intra-strain variation in animal susceptibility to infection. Limited side by side studies comparing the Cryptosporidium cell culture assay with the animal model have shown the two to produce comparable results in the case of C. parvum genotype 2 oocysts. Unlike the animal model, the Cryptosporidium cell culture assay is able to estimate infectivity of both C. parvum genotype 1 and genotype 2 oocysts.Of the approaches used to determine the infectivity of Cryptosporidum oocysts, the Cryptosporidium cell culture assay currently appears to be the most promising. However, at present there are a number of knowledge gaps associated with the Cryptosporidium cell culture assay which must be overcome before it can be used on a reliable, routine basis. The assay is not standardized as to the optimal cell line and culture medium formulation to be used. The sensitivity and specificity of the assay have yet to be determined in an impartial manner. Since a number of Cryptosporidium spp. including C. felis, C. canis, C. meleagridis, C. parvum genotype 1 and C. parvum genotype 2 now are known to infect humans the question is, will the Cryptosporidium cell culture assay work equally well for each of these different species? Once a standardized assay is available, then a laboratory round robin validation must be conducted to determine how robust the assay is. Incorporation of the Cryptosporidium cell culture assay into a complete method which would allow concentrating and purifying ocysts from various environmental matrices of varying turbidity must also be accomplished.

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Ware, M.W., Wymer, L., Lindquist, H.D.A., and Schaefer, III, F.W. Evaluation of Cryptosporidium oocyst recovery in water by EPA Method 1623 with a modified IMS dissociation procedure.. Presented at: International Symposium on Waterborne Pathogens, Lisbon, Portugal, September 22-25, 2002. 9/22/2002

Contact: Michael W. Ware

Abstract: EPA Methods 1622 and 1623 are the benchmarks for detection of Cryptosporidium spp. oocysts in water. 5-7 These methods consist of filtration, elution, purification by immunomagnetic separation (IMS), and microscopic analysis after staining with a fluorescein isothiocyanate conjugated monoclonal antibody and counterstaining with 4',6-diamidino-2-phenyl indole (DAPI).5-7 Studies evaluating the recovery of Methods 1622 and 1623 have demonstrated that recovery of oocysts is low and highly variable in real water samples, with the average oocyst recoveries all below 20% 1,3,4 Lindquist, et al. reported that only 30% of the recovered oocysts could be confirmed with DAPI stained nuclei.1 Reynolds, et al. developed a procedure which increased the rate of DAPI confirmation using a solid phase cytometer.2Study Objectives: Modifying treatment during and after IMS was evaluated to determine the effect on overall recovery and on the ability to confirm oocysts using DAPI staining.Experimental Conditions: Cryptosporidium parvum oocysts (Harley Moon strain) were purified by sieving, step gradients, and cesium chloride and used within 3 months of purification. All oocysts were stained with 1 x Crypt-a-glo or Aqua-glo (Waterborne, Inc.) and counterstained with DAPI (Sigma Chemical) at 0.4 ?g/ml. Briefly, stained oocysts were identified using the criteria in Method 1623.7 A FACS VantageSE (Beckton Dickinson) equipped with clone-cyt software was used for all flow cytometry analysis. Briefly during flow cytometry, a primary gate was drawn around the oocysts using forward (FSC) and side scatter (SSC). Stained oocysts were analyzed by FL1 (fluorescein isothiocyanate (FITC)) and FL5 (DAPI). Unstained, purified oocysts were prepared for experimental manipulations by sorting using the primary (FSC/SSC) gate into 1.5 ml tubes containing 1 ml reagent water.

Cryptosporidium parvum oocysts suspended in reagent water were used to compare DAPI stain reactions using two protocols. Control oocysts were suspended in reagent water. Heat processed oocysts were suspended in reagent water and heated for 10 minutes at 80 C, based on that described by Reynolds.2 The oocysts were then stained in suspension and examined by both flow cytometry and microscopy. IMS recovery experiments were performed in both reagent and river water to compare the IMS dissociation methods. Grab samples (>300 L) were taken from the Ohio River, filtered with Pall-Gelman Envirochek? filters, and eluted as described in Method 1623. These samples were pelleted, pooled into a packed pellet, and suspended in reagent water 5. Each IMS reaction was comprised of a 1 ml suspension containing either 150, 100, 50, 20, or 0 flow cytometry enumerated C. parvum oocysts in reagent water, 1 ml of reagent water was used to rinse the suspension tube, and either 8 ml of reagent water was added, or 8 ml river water suspension was added, representing approximately 0.4 ml of packed pellet. The IMS reactions were paired. IMS reagents were added and processed as described in Method 1623 5 through the point of aspiration of the supernatant after the IMS separation in the 1.5 ml tube. One set of sample was processed with acid dissociation. As described in Method 1623 5, 50 ?l of 0.1 N HCl was added to the oocyst-bead complex. This mixture was incubated for 10 minutes, and the oocysts were separated from the paramagnetic beads by exposure to a magnetic strip. The oocysts in solution were transferred to another 1.5 ml tube containing 5 ?l 1 N NaOH and 50 ?l reagent water. This set of samples was then heated for 10 min 80 C. One hundred ?l of reagent water was added to each tube in the other set. These oocysts were heated for 10 min at 80 C, separated from the paramagnetic beads by exposure to a magnetic strip, and transferred to another 1.5 ml tube. Both sets were then stained in solution, filtered through a 0.8 ?m porosity, 13 mm diameter polycarbonate filter, and enumerated by microscopy. Results:

The unheated oocysts had a very different DAPI profile than the heated oocysts when examined by flow cytometry. Over 99% of the nuclei from heat treated oocysts were stained by DAPI. Fewer than 4% of the unheated oocysts were stained. The microscopy results were similar. Exposure to 80 C did not effect either the antibody staining or the DIC appearance of the oocysts. Seeded reagent and river water samples were analyzed in parallel with either acid dissociation followed by heating or heat only dissociation. In both water types, heat dissociation improves mean oocyst recovery and DAPI confirmation. The mean oocyst recovery by acid dissociation in 48 reagent and river water samples was 28% ? 56 (? 2SD). The DAPI confirmation rate for these samples was 48% ? 64 In contrast, the mean oocyst recovery of 48 heat dissociated samples was 63% ? 28. The DAPI confirmation rate in these samples was 85% ? 26. The trends were similar in both water types, although the average oocyst recovery and DAPI confirmation rates were higher in reagent water samples. Relative differences in recovery between the two dissociation methods did not differ significantly by the number of oocysts present.Discussion: Water matrix particulates such as algae and cyanobacteria require that Cryptosporidium oocyst-like objects detected by fluorescent antibody be confirmed to prevent false positives. Method 1623 applies two confirmation methods: by demonstration of 1-4 sporozoites or by demonstration of 1-4 DAPI stained nuclei. Rarely are sporozoites visible by DIC microscopy. DAPI staining improves the confirmation rate, however, it remains quite low. This and other studies confirm that heating increases the DAPI confirmation rate.1,2 Furthermore, these DAPI stained oocysts are detectable by flow cytometry and they agree with microscopy results. This study demonstrates the utility of flow cytometry for the automated enumeration and confirmation of Cryptosporidium oocysts.

For both river and reagent water samples, the fraction of oocysts recovered was significantly greater in the samples processed with heat dissociation than by acid dissociation.

The mean oocyst recovery was nearly twice that of acid dissociation (p<0.0001, t-test), and the amount of variation decreased by nearly half (p<0.0001, f-test). The heat dissociated samples had nearly two-fold greater confirmation rate by DAPI. Since both samples were

Parshionikar, S., and Fout, G.S. Development of viral internal controls for the assessment of inhibitor removal from environmental water samples.. Presented at: 21st Annual Meeting of the American Society for Virology, Salt Lake City, UT, May 19-23, 2002. 5/21/2002

Contact: G. shay Fout

Abstract: The presence of human enteric viruses in drinking water is of public health concern. It is therefore important to be able to reliably detect these viruses in contaminating water sources. Cell culture methods are time consuming and also not all enteric viruses can currently be propagated in cell culture, e.g., caliciviruses. Molecular methods of detection such as RT-PCR are rapid and accurate but inhibitors present in environmental water samples can sometimes limit RT-PCR. For this purposel, amplification of internal controls for these viruses can be used to determine efficient removal of inhibitors from environmental samples. The efficiency of PCR is dependent on the length of the region to be amplified and the GC content. Since amplification obtained from an internal control that is significantly different in length and GC content may not be an accurate representation of PCR eficiency, we have developed internal controls for poliovirusus, HAV, Norwalk virus and rotavirus. These internal conrols have deletions of only a few basepairs compared to their wildtype sequences. Each viral internal control was developed such that it had the same primer binding sites as the virus. The probe region for the virus was modified with a new sequence. In particular, a deletion of a small number of viral bases was created and new bases were introduced keeping the GC content as close to the viral genome as possible. Each internal control RT-PCR band was only marginally smaller in length than its viral counterpart. Specifically, poliovirus was 9 bp, HAV was 15 bp, Norwalk was 8 bp and rotavirus was 6 bpsmaller than their respective viral RT-PCR fragments. The internal control RT-PCR bands could be separated from the viral RT-PCR bands by polyacrylamide gel electrophoresis. Each of the internal control DNA was sequenced to confirm the accuracy of the manipulated region. Results of hybridization experiments indicated that the internal control probes were specific and did not cross react with the viral probes and vice-versa. Thus, we have developed internal controls that can be used for assessing inhibitor removal from environmental samples.

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Seys, S.A., Mainzer, H.M., Heryford, A.G., Anderson, A.D., Fout, G.S., Sarisky, J.P., Musgrave, V., and Musgrave, K.J. Coordinating environmental public health practice with epidemiology and laboratory analysis: A waterborne outbreak of Snow Mountain virus in the Big Horn Mountains of Wyoming. Presented at: International Conference on Emerging Infectious Diseases, Atlanta, GA, March 24-27, 2002. 3/24/2002

Contact: G. shay Fout

Abstract: Background: In February 2001, the Wyoming Department of Health received reports of acute gastroenteritis among persons who had recently been on a snowmobiling vacation in the Big Horn Mountains. Initial interviews and laboratory testing suggested that exposure to a calicivirus in drinking water from a lodge was responsible for the illness.Methods: Environmental health specialists and epidemiologists from several state and federal agencies coordinated an investigation of environmental risk factors and system failures. The environmental assessment of the three lodges in the area included food service operations, water supply systems, and sewage disposal. A retrospective cohort study was conducted among 82 persons identified from guest registers to identify risk factors associated with illness. Stool and water system samples were collected for laboratory analysis.Results: Statistical analysis from the retrospective cohort study suggested that illness was associated with water consumption at one lodge (RR=3.3, 95% C.I.=(1.4, 7.7)). A chi-square test for linear trend showed that risk of illness increased significantly with the number of glasses of water consumed (p=0.0003). The consumption of individual food items was not statistically associated with illness. Reverse transcription polymerase chair reaction (RT-PCR) testing on 13 stool samples yielded 8 positives for Norwalk-like virus (NLV) genotype II. Fecal contamination of one of three operating wells was also found and one of the samples tested positive for NLV genotype II. The environmental assessment of the property revealed that an inadequately installed sewage system was delivering effluent into shallow soil with poor filtering capacity. This effluent likely contaminated drinking water of an overloaded water system.Conclusion: This event represents the largest waterborne outbreak ever reported in Wyoming. It illustrates the potential for waterborne transmission of viral gastroenteritis and the advantages of coordinating environmental public health practice with traditional epidemiologic and laboratory investigations. It is essential that personnel representing each of these entities participate fully in outbreak investigations and that environmental health specialist receive training in systems analysis and sampling methodologies. Outbreak investigations should address all of the systems of a facility including food service, water supply, and sewage systems.

Ruecker, N. J., Fout, G.S., Peterson, H. G., Watson, S., Lawrence, J., Appleyard, G., and Christofi, N. The use of RT-PCR for the detection of enteric viruses in prairie surface drinking water supplies. Presented at: 37th central Canadian Symposium on Water Pollution Research, Burlington, Ontario, February 4-5, 2002. 2/4/2002

Contact: G. shay Fout

Abstract: Concerns over the microbial safety of drinking water supplies have focused on bacteria and parasites while the occurrence of pathogenic waterborne viruses have been largely ignored. In fact, water supplies are not routinely monitored for human enteric viruses. This is despite the fact that pathogenic viruses are estimated to acccount for more than half of waterborne diseases. Reverse transcriptase polymerase chain reaction (RT-PCR) is used widely to detect viruses in clinical samples, but the application of RT-PCR to source-waters is compounded by two major factors. Relative to clinical samples, these water contain low numbers of pathogenic viruses and contain substances which are inhibitory to RT-PCR reactions. These substances inevitably become concentrated along with the virus particles. Without purification of the target viruses to remove inhibitors, false negatives are likely to occur, with serious health implications. We tested an RT-PCR method by screening four prairie surface water supplies for the presence of major enteric viruses (enterovirus, reovirus, rotavirus, hepatitis A virus and calicivirus). At the same time we tested for the presence of other microbes, and measured a range of water quality parameters. The presence of coliform bacteria, high particle and turbidity levels as well as dissolved inorganic and organic material indicate that these source waters were poor quality. No viruses were detected in the samples. However, the same samples seeded with virus also resulted in a few confirmed positive results. We argue that there is clearly a critical need to develop methods which are robust, that can be used to detect waterborne viruses with confidence, particularly for waters of poor quality. This is particularly important as global population density, intensive livestock farming and water usage increase.

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Ruecker, N. J., Lawrence, J., Peterson, H. G., Fout, G.S., Appleyard, G., and Christofi, N. The significance of enteric viruses and waterborne illness. Presented at: 10th National Conference and 1st Policy Forum on Drinking Water, Halifax, Nova Scotia, April 27-30, 2002. 4/27/2002

Contact: G. shay Fout

Abstract: With growing concern over drinking water safety, considerable attention has been directed towards microbial pathogens in source waters, and the adequacy of current methods used to detect, monitor and treat for these pathogens. The focus has been on bacterial and protozoan pathogens such as E. coli and Cryptosporidium, when in fact, pathogenic viruses are estimated to account for more than half of all waterborne disease outbreaks. This is significant because, despite recent advances in this field, routine monitoring of human viruses in water supplies is essentially non-existent, for several reasons. Viruses are by far the most difficult group of pathogens to detect and confirm in source water. Use of available methods is limited by prohibititive cost and lack of facilities and trained personnel. Current molecular methods used to detect, identify and confirm viruses from clinical samples cannot be applied directly to most source waters. Due to the low infectious dose of many human enteric viruses, the application of these methods to source waters is limited by the need to concentrate virus from large sample volumes (up to 200L) of water. Organic and inorganic components which tend to inhibit molecular viral detection methodology are typically present in source water and co-concentrate along with virus. The presence of virus should be of particular concern in small communities with poor source waters and rudimentary or inadequate treatment. It is also thought that the risk increases in high density urban areas, since many wastewater treatment processes reduce, but do not completely inactivate viral pathogens. The result is that many illnesses are not traced to a drinking or recreational water source, and thus the true impact of viruses on human health is unknown. A crucial need exists to: i) develop feasible and effective detection and monitoring methods, ii) use these to estimate viral pathogen occurrence and frequency in water supplies in order to better understand outbreaks and iii) develop strategies for the control of these pathogens.

Lindquist, H.D.A. Emerging waterborne pathogens. Presented at: 52nd Annual KU Environmental Engineering Conference, Kansas City, KS, February 5-6, 2002. 2/5/2002

Contact: H. d. alan Lindquist

Abstract: Water treatment and wastewater disposal practices have reduced the death rate from waterborne illness. However, the number of individuals made ill, and the number of outbreaks of waterborne disease still fluctuates greatly. This is partly due to newly recognized disease agents, that challenge water treatment systems in new ways. These disease agents are collectively called emerging diseases. Potentially emerging disease agents fit into all categories, viruses (calicivirus, astrovirus, and rotavirus), bacteria (Aeromonas spp., non-tuberculous Mycobacterium spp., and Helicobacter pylori), and protozoa (microsporidia, Cyclospora cayetanensis, and Toxoplasma gondii). It is not possible to predict which disease agent will cause the next large outbreak. However, a survey of these agents and the challenges they pose to water treatment, demonstrates that a well planned multi-barrier approach is the best route to protect public health in the face of these and other emerging diseases.

Schaefer, III, F.W. Laboratory guidelines for analysis of bioterrorism samples. Presented at: International Symposium on Waterborne Pathogens, Cascais, Lisbon, Portugal, September 22-25, 2002. 9/22/2002

Contact: Frank W. Schaefer

Abstract: After the attack on the World Trade Center on September 11, 2002, and the subsequent deaths associated with Bacillus anthracis spore contaminated mail, a worldwide need was apparent for increased laboratory capacity to safely analyze bioterrorism samples. The U.S. Department of Health and Human Services has furnished guidelines for microbiological and biomedical laboratory safety. These guidelines encompass laboratory practices and techniques, facility design, safety equipment, monitoring the analyst's health, vermin and insect control, as well as government control of select agents and specialized reagents.

Before work is initiated, the laboratory must have protocols which cover all standard operating procedures, quality assurance, chain of custody, and a detailed biosafety plan. Special emphasis is placed on the approach used in the event of either an accidental spill or accidental exposure. Extensive training in all aspects of the protocols is required for each analyst. Great care also is used to document both how and who processed the samples in case they have forensic significance. Design of the laboratory facility centers around containment and segregation of the sample analyses, so as few people as possible are involved. Persons who are immunocompromised should not under any circumstances be permitted access to such a hazardous facility. Key card access through an airlock allowing only authorized personnel into the laboratory helps ensure such a policy. All laboratory benches must have impervious surfaces. The walls and floors also should be sealed, so liquids cannot penetrate them. The laboratory equipment and benches should be set up in a fashion to allow routine cleaning and disinfection. There must be a sink in each laboratory to facilitate hand washing. Interlocking double door autoclaves, a specialized negative pressure ventilation system, and waste stream treatment must all be part of the design. The required safety equipment depends upon the type of analysis being performed. Generally gloves, laboratory coat or gown, shoe covers or boots, and eye protection are required. If the protocol entails analysis of a sample that is prone to the production of an aerosol, then a respirator is mandated. Most if not all procedures should be conducted in a biosafety cabinet. When centrifugations are necessary, they should be done with capped containers that in turn are placed in safety centrifuge cups. These cups are designed to prevent aerosols from being released during centrifugation. A biohazard warning sign naming the organism and incorporating the international biohazard symbol must be posted on all laboratory doors. A key portion in the operations of any well managed laboratory is monitoring the health of the workers. Baseline and periodic serum samples should be collected and stored for future reference. Daily body temperature monitoring is encouraged. If available, vaccination should be offered. In addition, a treatment plan in case of exposure and/or infection is mandatory.

The ultimate responsibility for the safe operation of the laboratory falls to the director. He or she must establish policies and procedures that fully inform the analysts regarding all potential hazards. The director must review all protocols for scientific completeness and safety. Furthermore, the performance of each analyst must be reviewed and certified periodically. Use of independently prepared performance evaluation samples and external audits are strongly encouraged for this certification process. In the event a particular analyst does not meet the established criteria, then it is the director's responsibility to see corrective action is taken immediately. Moreover, the director must make sure all laboratory activities comply with federal and state regulations.

Lindquist, H.D.A., Varma, M., Ware, M.W., Aranzamendi, C., and Vargas, C. Comparison of microscopic versus molecular diagnosis of Cyclospora cayetanensis. Presented at: Joint meeting of the International Congress of Parasitology and Annual Meeting of the American Society of Parasitologists, Vancouver, British Columbia, Canada, August 4-10, 2002. 8/4/2002

Contact: H. d. alan Lindquist

Abstract: Objective: to investigate several ways to diagnose the food and waterborne protozoan parasite Cyclospora cayetanensis. Cyclospora cayetanensis is a protozoan parasite that infects human beings and causes gastroenteritis. Diagnosis of this parasite is complicated by the fact that it is difficult to stain, or visualize through the techniques associated with typical ova and parasite examinations. Materials and Methods: More than one hundred stools were collected from children in Lima Peru during the course of normal diagnostic and treatment activities. These stool samples were screened by standard ova and parasite evaluation. The remainder of those samples that were found to be positive for parasites were screened again, using fluorescent microscopy, to detect any autofluorescent parasites, which were then confirmed as to genus or presumptively to species using differential interference contrast microscopy. These results were recorded. Aliquots of samples were removed for analysis by taqman assay. This assay incorporates PCR using a dual labeled fluorescence probe designed to increase in fluorescence intensity as it is digested during a successful PCR. Results: Results of this assay were compared to the results from microscopic examination of the specimens. It was found that both microscopic analysis and the PCR based assay were able to detect Cyclospora cayetanensis oocysts, while standard ova and parasite examination was not.Conclusions: Standard ova and parasite examinations are not sufficient to detect this emerging infection. Both fluorescence microscopy and a PCR based method succeed in detecting this parasite when standard methods did not. The PCR based assay provided a quantitative capability, but the accuracy of this quantitation was not tested in this study.

Lindquist, H.D.A. Special pathogens/bioterrorism capabilities within NERL microbiology. Presented at: EPA Regional Science and Technology Officers, Washington, DC, May 1, 2002. 5/1/2002

Contact: H. d. alan Lindquist

Abstract:

Schaefer, F.W. Method 1623 for detecting giardia and cryptosporidium. Presented at: International Symposium on Waterborne Pathogens, Cascais/Lisbon, Portugal, September 22-25, 2002. 9/22/2002

Contact: Frank W. Schaefer

Abstract:

Schaefer, F.W. Regulation of drinking water pathogens in the U.S.. Presented at: International Symposium on Waterborne Pathogens, Cascais/Lisbon, Portugal, September 22-25, 2002. 9/22/2002

Contact: Frank W. Schaefer

Abstract:

Lindquist, H.D.A. Anthrax remediation research needs. Presented at: 4th Decontamination Commodity Area Conference (DECON 2002), San Diego, CA, October 22-24, 2002. 10/22/2002

Contact: H. d. alan Lindquist

Abstract: The Environmental Protection Agency has initiated a research program to respond to the immediate needs arising from the recent Bacillus anthracis bioterrorism events. Although the program has a strong emphasis on anthrax, other pathogens and chemical agents, including toxic industrial chemicals are also addressed. This program of research includes five technical areas: detection; containment; decontamination; disposal; and risk assessment, communications, and technology transfer. Detection research will address methods to detect biological and chemical agents to determine if an attack has occurred, and to guide disinfection/decontamination operations. Containment encompasses research to prevent or limit the damage from indoor releases of biological or chemical agents. Decontamination research focuses on disinfection of B. anthracis spores, as well as other hazardous biological or chemical agents. Disposal research will focus on safe packaging, transport, and disposal of the waste streams and disinfection byproducts resulting from disinfection or decontamination activities. Risk assessment, communications, and technology transfer projects are designed to provide methods to assess and communicate risk and to communicate new methods and improved technologies to users. Anticipated products are all planned to be available for field use in the near term and include guidelines, standardized and tested procedures, and technology evaluations. Customers of these products include early responders, field decontamination personnel, architects, and building owners and managers. The research will be carried out predominantly through extramural agreements and partnerships with both public and private entities; as well as utilizing U.S. EPA in-house capabilities where they already exist. Some projects within this strategy are currently being implemented, while others are awaiting final approval through the peer review process.

Ware, M.W., Schaefer, III, F.W., Lindquist, H.D.A., and Wymer, L.J. Cryptosporidium oocyst recovery in water by EPA method 1623: Evaluation of a modified IMS dissociation. Presented at: Ohio River Basin Consortium for Research and Education, Highland Heights, KY, November 6-7, 2002. 11/6/2002

Contact: Frank W. Schaefer

Abstract: EPA Methods 1622 and 1623 are the benchmarks for detection of Cryptosporidium spp. oocysts in water. These methods consist of filtration, elution, purification by immunomagnetic separation (IMS), and microscopic analysis after staining with a fluorescein isothiocyanate conjugated monoclonal antibody and counterstaining with 4',6-diamidino-2-phenyl indole (DAPI). Studies evaluating Methods 1622 and 1623 have demonstrated that oocyst recovery is low and highly variable in real water samples. In addition, the confirmation of oocysts is rare. Confirmation is vital to prevent false positive results because some algae can appear to be oocysts.This study developed an alternative IMS dissociation procedure using heat instead of acid. This modification was evaluated for recovery and DAPI confirmation by performing IMS experiments of seeded reagent and Ohio River water samples which were analyzed in parallel with either acid or heat dissociation.In both water types, heat dissociation improved mean oocyst recovery and DAPI confirmation. The mean oocyst recovery by acid dissociation in 48 reagent and river water samples was 28% 1 28 (1 SD). The DAPI confirmation rate for these samples was 48% 1 32 In contrast, the mean oocyst recovery of 48 heat dissociated samples was 63% 1 14. The DAPI confirmation rate in these samples was 85% 1 13. The trends were similar in both water types, although the average oocyst recovery and DAPI confirmation rates were higher in reagent water samples. The mean oocyst recovery was nearly twice that of acid dissociation ($p<0.0001$, t-test), and the amount of variation decreased by nearly half ($p<0.0001$, f-test). The heat dissociated samples had nearly two-fold greater confirmation rate by DAPI. The performance of Method 1622/3 could be improved by changing the IMS dissociation procedure from acid to heat which would allow for greater oocyst recovery and confirmation.

Jan 1, 2002 - Dec 31, 2002

Presented Published

Lindquist, H.D.A. The increasing need for surveillance: Water sources and environmental factors. Presented at: Symposium 30: Emerging Food- and Water-borne Protozoan Parasites. 51st Annual Meeting of the American Society of Tropical Medicine and Hygene, Denver, CO, November 10-14, 2002. 11/10/2002

Contact: H. d. alan Lindquist

Abstract: For the Symposium 30: Emerging Food- and Water-borne Protozoan Parasites. 51st Annual Meeting of the American Society of Tropical Medicine and Hygiene.Denver, Colorado, November 10-14, 2002The outbreaks of giardiosis in the 1980's and the outbreak of cryptosporidiosis in Milwaukee, have demonstrated the susceptibility of public water supplies to contamination with protozoan parasites. Many protozoan parasites are resistant to the levels of residual disinfectant remaining after water treatment. Therefore, if parasitic transmission stages are passed through an initial treatment system, the remaining disinfectant residual may not be sufficient to ensure public safety. Thus, it is imperative that treatment be matched to the severity of the contamination of the source water. Highly contaminated source water must be treated in a manner that is efficacious in inactivating and removing protozoan transmission stages. To determine the level of contamination of source water, several methods have been used or proposed. In addition, the list of protozoa to be detected is also growing, as the incidence of disease from waterborne transmission of a variety of protozoa increases. The methods used to detect protozoa in water have included the Information Collection Rule Method and EPA Method 1623. Other technologies that are being investigated for the detection of protozoa in the environment include molecular methods such as fluorescent in situ hybridization probes, and polymerase chain reaction based assays.

Creed, J.T., Gallagher, P.A., Schwegel, C.A., Parks, A., and Gamble, B.M. EPA studies of arsenic speciation in seafood matrices with an emphasis on extractability and arsenosugar integrity. Presented at: 2002 Pittsburgh Conference, New Orleans, LA, March 17-22, 2002. 3/17/2002

Contact: John T. Creed

Abstract: The anthropogenic and geological occurrence of arsenic (As) results in human exposure to a potentially carcinogenic element. The two predominant pathways to As exposure are drinking water (DW) and dietary ingestion (DI). DW exposures are almost exclusively toxic inorganic As. This toxicity has led to the reformulation of the MCL for As and increased the number of DW sources which requires some type of treatment. The effectiveness of this treatment can be influenced by the oxidation state of the inorganic As present in the water. Therefore, deciding which treatment to use often requires the inorganic As be speciated (separated) into As(III) and As(V). The preservation of this natural distribution during shipment can be problematic, especially in iron rich waters. The first part of this presentation will describe a systematic evaluation of factors which can influence the preservation of As. An emphasis will be put on the use of the EDTA in combination with acetic acid to stall or inhibit the iron precipitation reactions which produce "FeOAs"solids. Five DWs will be evaluated and the stability of their native As distribution in time will be discussed. The second major source of As exposure is DI, with seafood representing over 50% of the total dietary exposure. From a total metal perspective, dietary As exposures can easily exceed those from DW, especially in populations with high seafood consumption rates. Unlike DW, dietary As is a mixture of toxic and non-toxic arsenicals [e.g., AsB, arsenosugars, etc.]. For this reason, to assess toxicity from dietary exposures, it is necessary to speciate the As. The extraction of arsenicals from solid seafood matrices gives rise to concerns regarding the species specific stability and the need to minimize spcies specific extraction bias. However, there is uncertainty as to whether or not the native arsenical distribution in the matrix reflects what elutes from the chromatographic column, Aggressive extraction condidtions can lead to a more quantitative removal but may produce arsenosugar degradation products. This presentation will provide some preliminary data indicating that some arsenosugars tend to bind to secondary substrates making them difficult to chromatograph by conventional anion IC. Most of the results will be collected using IC-ICP-MS, and IC-ESI-MS/MS will be used in an attempt to provide structural informatoin on degradation products.

Jan 1, 2002 - Dec 31, 2002

Presented Published

Gamble, B.M., Gallagher, P.A., Shoemaker, J.A., Heck, A., Freeman, D.M., Schwegel, C.A., and Creed, J.T. Investigation of arsenosugar-substrate interactions in seafood extracted with tetramethylammonium hydroxide. Presented at: 2002 Winter Conference on Plasma Spectrochemistry, Scottsdale, AZ, January 6-12, 2002. 1/6/2002

Contact: John T. Creed

Abstract: The maximum contaminate level (MCL) for arsenic is currently being revised within the U.S. Safe Drinking Water Regulations. The proposed MCL is 10 ng/g. The formulation of this MCL is influenced by a wide variety of factors including risk assessments based on health data, best available treament technology, analytical monitoring capability and relative exposure source estimates. The relative exposure source estimate is formulated to assess the exposure from all possible routes. For arsenic, the two major exposure routes are dietary and drinking water ingestion. The uncertainty associated with dietary exposure source estimates is relatively high because species specific data on target foods is limited and poor extraction efficiencies in certain seafood samples makes species specific risk assessment difficult. The uncertainty generated by poor extraction efficiencies can be minimized by using a more aggressive extraction solvent, such as tetramethylammonium hydroxide. When a more aggressive technique is employed, a larger percentage (90+%) of the available arsenic is liberated into solution from the solid seafood matrices. The resulting solution contains "substrates" which can bind native arsenosugars and result in a "substrate-arsenosugar" complex that is unchromatographable using anion exchange chromatography. Currently, the substrate is thought to be a protein or lipid with a molecular mass of less than 10 kDa. In addition, the resulting complex is acid-labile at pH 4. This poster will investigate the nature of the arsenosugar-substrate interaction and attempt to identify the substrate using size exclusion chromatography with ICP-MS and ESI-MS/MS detection. By better understanding the arsenosugar-substrate interaction, a more quantitative, species-specific arsenic risk assessment can be established for dietary exposure in seafood samples.

Talley, J.M., Shoemaker, J.A., and Fout, G.S. Strain differentiation and determination of Capsid proteins of Coxsackievirus by MALDI-MS. Presented at: American Society for Mass Spectrometry, Orlando, FL, June 2-6, 2002. 6/2/2002

Contact: Jody A. Shoemaker

Abstract: Introduction: Contamination of viruses in water environments (rivers, lakes, sources of drinking water) is a new threat and serious health problem. Amongst organisms discharged from sewage septic systems is the coxsackievirus (single-stranded RNA virus). Differentiation between different strains of the coxsackievirus using conventional biological methods takes up to several months. The capsid protein sequences of several coxsackie strains are known and therefore amenable to rapid analysis by matrix-assisted laser desorption (MALDI) mass spectrometry (MS). This study represents the first investigation of the four capsid proteins of coxsackie B1, B3, B5 and B6 viruses by MALDI-M.Methods and Instrumentation: Virus purification consisted of underlaying the viral sample with sucrose and pelleting the virus through the sucrose pad at 10 degrees C for two hours. The virus was layered atop a 7.5-45% sucrose gradient, centrifuged at 10 degrees C for 1.5 hours and fractionated. The virus was then layered atop a 20-45% CsCI gradient and centrifuged at 10 degrees C overnight. The virus was collected and desalted with a microcon-100 concentrate. The viral sample was reacted with 0.5% Trifluroacetic acid (TFA) in water and mixed with a matrix consisting of a 70/30 acetonitrile in 0.1% TFA water. Acid-resistant Lab Label Protection Tape was applied to the target and acted as a hydrophobic surface. MALDI-MS experiments were performed on a Bruker BIFLEXIII. Thermal relaxation of the excited matrix resulted in absorption of the capsid protein into the gas phase. All capsid proteins were run in the linear mode and detected in the first detector by their mass-to-charge ratio.Preliminary Data: Reacting the virus with TFA disrupted the protein capsid and released the four viral proteins (VP1, VP2, VP3 and VP4). Preliminary results from the capsid protein of the coxsackie B6 virus show four peaks corresponding to the four viral proteins, m/z 31,549 (VP1), m/z 14,317 (VP2[H]2+), m/z 13,047 (VP3[H]2+) and m/z 7,528 (VP4). The four capsid proteins from the coxsackie B5 virus were also easily identified and correspond to VP1 (m/z 33,274), VP2[H]2+ (m/z 13,801), VP3[H]2+ (m/z 13,699) and VP4 (m/z 6,939). The results thus far show that the differentiation between strains of coxsackievirus can be achieved by MALDI-MS. The information we obtained will aid microbiologists in devleoping a more rapid method of identifying viruses in drinking water.

Hu, Y., Pellizzari, E.D., Akland, G.G., Melnyk, L.J., and Berry, M. Use of pharmacokinetic modeling for dietary exposure study design and model evaluation. Presented at: ISEA Annual Meeting, Vancouver, British Columbia, August 11, 2002. 8/11/2002

Contact: Lisa J. Melnyk

Abstract: It has been shown that young children have potentially higher dietary pesticide intake when they handle foods while eating. Foods become contaminated with pesticides from "dirty" hands and home surfaces. A dietary intake model has been developed to estimate the potential increase in pesticide exposure caused by food handling and the model prediction has been confirmed in the small-scale field study. However, modelers often struggle with the validity of models and often model validation using biomarkers is unsuccessful because of large intra- and inter- individual variabilities for exposures, metabolism, physiological parameters, multiple sources of the exposures, and laboratory measurement errors. In this work, we demonstrated how to efficiently conduct a field study with the appropriate study design guided by pharmacokinetic modeling to minimize these problems. We used the children's dietary exposure model to evaluate increased pesticide intakes. Our work indicated that a longitudinal study in which the subject can serve as his/her own control, proper selection of pesticides with a biological half-life in appropriate range, and homes with pesticide loadings at specified levels were important factors to design a successful field study to evaluate the children's dietary intake model. We also explored the effects of other exposure pathways such as exposures via inhalation and indirect ingestion from non-dietary sources to evaluate the model using urinary biomarkers. We attempted to evaluate the dietary pesticide intake model using two methods: the left-over foods and urinary measurements in a small-scale longitudinal study that involved 3 young children. Exposed days that allowed normal dietary exposure caused by handling of the foods alternated with unexposed days to create a predicted zig-zag pattern in the urinary pesticide metabolite measurements. The left over food measurements demonstrated that the handled foods indeed had much higher pesticides than foods that were not handled by the children (P=0.007). The zig-zag pattern predicted by toxicokinetic modeling was also observed in actual urinary metabolite measurements of all of the 3 subjects, which indicates an observable increase in dietary pesticide intake caused by children's handling of the foods. Comparison of the predicted urinary measurements and actual measurements, however, indicated model over prediction or unmeasured exposure sources. Nonetheless, our work suggested substantial amounts of dietary pesticide intake resulting from young children's handling of the food that should not be neglected and the model provided a sound base for further fine-tuning.

Hu, Y., Akland, G.G., Pellizzari, E.D., Melnyk, L.J., and Berry, M. Conceptual framework for categorizing young children's eating behaviors. Presented at: ISEA Annual Meeting, Vancouver, Canada, August 11, 2002. 8/11/2002

Contact: Lisa J. Melnyk

Abstract: Recent studies of total dietary ingestion of common indoor contaminants have demonstrated that young children's behaviors while eating can lead to a significant source of food contamination. The difference between children eating their food items with or without their hands which have come into contact with indoor surfaces contaminated by pollutants has been shown to be potentially significant, but has not yet been fully quantitated. Food items that are eaten with utensils will have less surface-to-food or surface-to-hand-to-food pesticide contamination compared to food items that are eaten with contaminated hands in contaminated environments. It has been observed from a recent field study that "messy" eaters have higher levels of contaminated food and urinary biomarker levels of diazinon as compared to "neat" eaters. "Messy" eaters had higher surface-to-food or surface-to-hand-to-food contact frequencies for most food items which resulted in higher levels of contaminated leftover food and higher urinary biomarker levels of the chemical metabolite under study. Determining the activity factor to categorize eating behaviors is one objective of an ongoing study to accurately model dietary exposure of young children. An intermediate objective is to use a surrogate food to measure hand-to-food transfers of pesticides to determine the effect of multiple touches. This will also be used to determine the activity factors that will distinguish three categories of eating behaviors. Three categories of eaters, i.e., neat, messy, and in-between, are determined by analyzing videotapes of children eating. Statistical methods such as tree-based cluster analysis are used to identify other factors that affect children's eating behavior, including the nature of the food items, meal, children's age, gender, and general hygiene practices. Once these factors are identified, the ultimate goal will be to predict total ingestion for a child based on a dietary model, by collecting measurements of the contaminant on selected surfaces of the home and observations of the child's eating behaviors with surrogate food items. Pesticides will be measured from the surrogate food to determine transfers from surfaces and hands to the item to attempt to verify the categories of eaters.

Jan 1, 2002 - Dec 31, 2002

Presented Published

Potter, B.B., and Wimsatt, J. C. Investigation of response differences between different types of total organic carbon (TOC) analytical instrument systems. Presented at: ACS Meeting, Orlando, FL, April 11-15, 2002. — 4/11/2002

Contact: Billy B. Potter

Abstract: Total organic carbon (TOC) and dissolved organic carbon (DOC) have long been used to estimate the amount of natural organic matter (NOM) found in raw and finished drinking water. In recent years, computer automation and improved instrumental analysis technologies have created a variety of TOC instrument systems. However, the amount of organic carbon (OC) measured in a sample has been found to depend upon the way a specific TOC instrument treats the sample and the way the OC is calculated and reported. Specifically, relative instrument response differences for TOC/DOC, ranging between 15 to 62%, were documented when five different source waters were each analyzed by five different TOC instrument systems operated according to the manufacturer's specifications. Problems and possible solutions for minimizing these differences are discussed.

Potter, B.B. Investigation of response differences between different types of total organic carbon (TOC) analytical instrument systems. Presented at: American Chemical Society Spring Meeting, Orlando, FL, April 7-11, 2002. — 4/7/2002

Contact: Billy B. Potter

Abstract: Total organic arbon (TOC) and dissolved organic carbon (DOC) have long been used to estimate the amount of natural organic matter (NOM) found in raw and finished drinking water. In recent years, computer automation and improved instrumental analysis technologies have created a variety of TOC instrument systems. However, the amount of organic carbon (OC) measured in a sample has been found to depend upon the way a specific TOC instrument treats the sample and the way the OC is calculated and reported. Specifically, relative instrument response differences for TOC/DOC, ranging between 15-62%, were documented when different source waters were each analyzed by five different TOC instrument systems operated according to the manufacturer's specifications. Problems and possible solutions for minimizing these differences are discussed.

Melnyk, L.J., Berry, Jr., M.R., Tomerlin, J.R., Barraj, L., and Gordon, S.M. Alternatives to duplicate diet methodology. Presented at: ISEA Annual Meeting, Vancouver, Canada, August 11-15, 2002. — 8/11/2002

Contact: Lisa J. Melnyk

Abstract: Duplicate Diet (DD) methodology has been used to collect information about the dietary exposure component in the context of total exposure studies. DD methods have been used to characterize the dietary exposure component in the NHEXAS pilot studies. NERL desired to evaluate its current DD methodology to detemrine if alternative methods could provide 1) the same amount of information for less cost or 2) additional informaiton for approximately the same cost. Four alternatives were formulated for a public workshop to dscuss each in detail: 1-- the Cyclic Sub-Portion Duplicate Diet (CSPDD) is intended to greatly reduce the amount of material that is handled during the laboratory phase of a total exposure study. The CSPDD accomplishes this by requiring study participants to collect only a small sub-sample from each food consumed during the day instead of an exact duplicate portion. The CSPDD incorporates detailed food diaries and/or photographic records to record total amount of food consumed. The sampling procedure and subsequent sample analysis is repeated through multiple cycles. 2 -- The sub-Population Duplicate Diet (SPDD) combines a food frequency questionnaire administered to the entire sample population with a standard DD administered to a statistically representative sub-sample of the target population. 3 -- The Targeted Foods Duplicate Diet (TFDD) uses available information to identify "target" foods which are likely to contain the contaminants of interest. The targeted foods are collected separately from non-target foods, and detailed written or photographic records are kept for portion size and food consumption. 4 -- The Total Population Diet (TPD) preselects foods and beverages presumed to be associated with the contaminants of interest. Eligible households are identified, and prescreened by questionnnaire to determine which, of any, of the preselected foods they normally consume. Information is also obtained about the households typical diet. specific households are directed to prepare, eat and retain individual samples of selected target foods and beverages on the days of the field study. The foods consumed are weighed and the amounts are recorded, as is information about food preparation. Samples are composited according to food groupings (e.g., meats, vegetables) and in proportion to reported portion size, so that relative contribution of specific food groups is possible. The public workshop included the convening of a panel of experts to offer recommendations about which method, if any, to investigate further. In general, the workshop consensus was the current DD methodology has worked well. The workshop thought that the CSPDD alternative might be worth investigating further because of its potential to significantly reduce study costs. The workshop also concluded that the TFDD might have some utility, but only under certain specific study conditions.

Jan 1, 2002 - Dec 31, 2002

Presented Published

Bernard, C.E., Melnyk, L.J., and Berry, Jr., M.R. Measurements of transferrable residue from ceramic tile, vinyl tile, hardwood flooring, and carpet using a press sampler and C18, PUF, and cotton sampling disks. Presented at: ISEA Annual Meeting, Vancouver, Canada, August 11, 2002. 8/11/2002

Contact: Lisa J. Melnyk

Abstract: Unintentional and avoidable human exposure is a consequence of pesticide use indoors. Pesticides on household surfaces are a source of exposure to children. Therefore, concern has been raised regarding the potential for contamination of foods in homes where pesticides have been used. The exposure pathways that contribute to total dietary intake of pesticides for infants and children are believed to be different than that of adults. This includes indirect dietary exposure through either direct contact between foods and contaminated surfaces (surface-to-food transfer) and/or through an intermediate, such as hands (surface-to-hands-to-food transfer). Accurate measurements of surface residue transfer are needed to determine the extent of pesticide transfer to foods handled and/or eaten by a child. Consequently, a method is needed to meausre pesticide residues available on surfaces. Wiping surfaces with a solvent moistened cotton gauze has been an accepted method for measuring surface residues, but the use of solvents can mar the sampled surface, which would be an unacceptable outcome for field sampling. Alternatively, a surface press sampler that presses C18 sampling disks (3M TM Empore TM Extraction disks composed of 90% Octadecyl adsorbent particle and 10% inert PTFE) against a surface for a specified period of time with a constant applied force has been used to measure transferable surface residue. The C18 disks, which adsorb and/or absorb the residue during contact with the surface, are removed, extracted in solvent and analyzed for the transferred surface residue concentration. The objective of this study was to determine if a surface press sampler in conjunction with either dry preconditioned C18 polyurethane foam (PUF; 1/4" thickness) or cotton (100% natural cotton) disks could be used to obtain transferable residue information from household surfaces. The extent of surface residue transfer to the C18, PUF and cotton dosimeter disks were then compared to isopropanol surface wipes. The surfaces tested consisted of ceramic tile, vinyl tile, hardwood flooring, and carpet. Each surface was contaminated with an aqueous solution of pesticides commonly used and/or found in homes (diazinon, malathion, chlorpyrifos, fipronil, cypermethrin, deltamethrin, cyfluthrin and permethrin) at loading rates of 0.1 and 1 ug/cm2, which are similar to previously measured deposition levels reported from residential monitoring of broadcast and total release aerosol applications. Following spraying, each surface was allowed to dry then duplicate contaminated surfaces were press sampled for surface contact times of 2,5,10, and 60 min. The extent of residue transfer to C18, PUF and cotton disks were compared and will be reported as percentages of pesticides transferred based on wipes. Future studies will include comparing the extent of residue transfer to the disks with that of pesticide transfer to food items.

Creed, J.T., Gallagher, P.A., Shoemaker, J.A., Schwegel, C.A., Gamble, B.M., and Parks, A.N. Arsenic speciation in problematic seafood matrices: the importance of a species specific mass balance. Presented at: International Conference on Arsenic Exposure and Health Effects (SEGHA), San Diego, CA, July 14-17, 2002. 7/14/2002

Contact: John T. Creed

Abstract: Arsenic has two major exposure routes: dietary and drinking water ingestion. Dietary exposures can easily exceed those typically associated with drinking water but the risk associated with these exposures is strongly influenced by the toxicity of the arsenicals present in the sample. For instance, a major source of dietary arsenic is seafood but 90+% of the "extractable" arsenicals can be non-toxic; therefore, species specific information is essential in estimating the risk associated with dietary exposures. A source of uncertainty associated with estimating dietary risks is the limited availability of species specific data on target foods.One of the analytical problems associated with arsenic speciation (species specific detection) in dietary samples is the need to extract the arsenicals from the solid dietary matrix. In many cases, the predominant "extractable" arsenical associated with seafood has been non-toxic arsenobetaine, but in certain seafood matrices the "extractable" arsenicals may be less than 50% of the total arsenic. This raises questions about the toxicity of the "unextractable" arsenicals and the potential for underestimating the risk (i.e., exposure) based on this analytical extraction bias. These low extraction efficiencies can be further complicated by unchromatographable arsenicals. The net result is the unextractable and unchromatographable fraction sequentially decrease the available speciation information. This presentation will focus on the use of tetramethylammonium hydroxide as an extraction solvent in seafood samples collected in the Pacific Northwest clams, mussels, and oysters). This presentation will address the conversion of unchromatographable species to chromatographable arsenosugars and how this influences the overall speciation recovery and in turn the ability to assess the risk from these matrices.

Shoemaker, J.A. Method development for Alachlor ESA and other acentanilide herbicide degradation products. Presented at: 224th American Chemical Society National Meeting, Boston, MA, August 18-22, 2002. 8/18/2002

Contact: Jody A. Shoemaker

Abstract: Introduction: Acetanilide herbicides are frequently applied in the U.S. on crops (corn, soybeans, popcorn, etc.) to control broadleaf and annual weeds. The acetanilide and acetamide herbicides currently registered for use in the U.S. are alachlor, acetochlor, metolachlor, propachlor, flufenacet and dimethenamid. Acetanilide degradation products are generally more water soluble and mobile than the parent herbicide, thus there is greater potential for these degradates to be found in ground waters and surface waters. The most common acetanilide degradation products are the ethanesulfonic acid (ESA), oxanilic acid (OA) and sulfinylacetic acid (SAA) derivatives of the parent herbicides. The ESA and OA degradates of alachlor, metolachlor, and acetochlor have been reported in U.S. Midwestern surface and ground waters at typical concentrations of 0.1- 20 g/L [1-5]. High priority is given to this research project in EPA's Office of Research and Development because acetanilide degradation products are identified on the 1998 Drinking Water Contaminant Candidate List (CCL) [6]. Specifically, analytical methodology is needed to gather occurrence data on the acetanilide degradation products. While several methods have been reported in the literature [3,7], these methods do not address issues specific to analyzing compounds in drinking water, such as preservatives and internal and surrogate standards. In addition, the reported methods do not contain all the target analytes listed in Table 1. The objective of this research is to develop an accurate and precise analytical method to detect and quantitate the ESA, OA and SAA acetanilide pesticide degradates in drinking water matrices. This will include two steps: 1) evaluating the capability of solid phase extraction (SPE) techniques to concentrate the acetanilide ESA, OA, and SAA degradates from drinking water, and 2) evaluating the capability of liquid chromatography/mass spectrometry (LC/MS) techniques to separate and detect ESA, OA, and SAA acetanilide herbicide degradates in the concentrated sample extracts. Future occurrence data gathered with this developed method can then be used in determining whether to study the health effects of the acetanilide herbicide degradation products, and ultimately whether to regulate these compounds or remove them from the CCL. Materials and MethodsReagents/Standards. The ESA, OA, and SAA degradation products of alachlor and propachlor were obtained from Monsanto Co. (St. Louis, MO). The ESA and OA degradation products of metolachlor were obtained from Novartis (Greensboro, NC). The ESA and OA degradation products of dimethenamid were obtained from BASF Corp. (Research Triangle Park, NC) and the acetochlor ESA and OA degradates from Zeneca (Berkshire, UK). (Note: Novartis and Zeneca Crop Protection Units merged in 2001 to form Syngenta.) Flufenacet ESA and OA were obtained from Bayer Corp. (Stilwell, KS). The internal standard, 4-phenoxybenzoic acid (PBA), and surrogate, 2-benzoylbenzoic acid (BBA), were purchased from Sigma-Aldrich (Milwaukee, WI). The preservatives, copper(II) sulfate pentahydrate, tris(hydroxymethyl)nitromethane (trisnitro), diazolidinylurea (DZU), and trizma (tris(hydroxymethyl)aminomethane+tris hydrochloride) were purchased from Sigma-Aldrich and sodium sulfite from Fisher (Fair Lawn, NJ). Optima grade methanol and ACS grade ammonium acetate were purchased from Fisher. Deionized water was used from a four-stage Milli-Q water system (Millipore; Bedford, MA). Ten millimolar ammonium acetate was prepared by adding 0.77 g of ammonium acetate to 1 L of deionized water. The acetanilide, internal standard, and surrogate spiking mixes were prepared in methanol and calibration standards were prepared in the 10 mM ammonium acetate. Three or four point linear calibration curves were generated daily using PBA as an internal standard and BBA as the surrogate standard each at 200 pg/ L. Calibration points were generated at 10, 20, 50, 100, and 200 pg/ L. Solid phase extraction. Samples were extracted using a Zymark AutoTrace SPE WorkStation (Hopkinton, MA) and Supelco (Bellefonte, PA) ENVI-CARB carbon cartridges (6 mL, 250 mg). The deionized water samples were fortified with BBA at 200 pg/ L and the preservatives at the concentrations noted in Table 1. The carbon cartridges were conditioned with 20 mL of 10 mM ammonium acetate in methanol (prepared by adding 0.77g of ammonium acetate to 1 L of methanol) followed by 30 mL of deionized water. Water samples were passed through the cartridges at a flowrate of 10 mL/min. The target analytes were eluted from the carbon cartridges with 10 mL of 10 mM ammonium acetate prepared in methanol at a flowrate of 5 mL/min. All extracts were evaporated to dryness with a nitrogen stream in a 70 C water bath, spiked with 20 L of 10 ng/ L PBA and reconstituted to 1 mL with 10 mM ammonium acetate prepared in deionized water. Liquid chromatography/mass spectrometry. Extracts were analyzed on a ThermoFinnigan (San Jose, CA) LCQ Deca ion trap mass spectrometer equipped with an atmospheric pressure ionization source and an Agilent (Palo Alto, CA) HP1090 LC. The target analytes were quantitated by negative ion electrospray using the peak area of the [M-H]- for each target analyte. The sheath gas (80, unitless), auxillary gas (30, unitless), and heated capillary temperature (300?C) were optimized on m/z 314 of alachlor ESA (0.25 mg/L) infused at 0.4 mL/min. An Agilent Hypersil (2.1 x 100 mm, 5 m) C18 analytical column was used to separate the target analytes at a flowrate of 0.4 mL/min and column temperature of 70?C. The injection volume was 100 L. The binary mobile phase gradient composition was (A)10 mM ammonium acetate (ammonium acetate in deionized water, pH = 7.0 unadjusted) and (B) methanol. The initial mobile phase composition was 90:10 A:B, with a linear ramp to 80:20 A:B in 7 minutes followed by a linear ramp to 75:25 A:B in 3 minutes. The column was held at 75:25 A:B for 7 minutes and allowed to re-equilibrate to initial conditions for 15 minutes prior to the next injection.Results and DiscussionPrevious research has demonstrated chromatographic separation of 12 target acetanilide degradates, including chromatographic

separation of the alachlor/acetochlor ESA and OA structural isomers [8,9]. Flufenacet ESA, flufenacet OA, a potential internal standard, and a surrogate standard have been added to the analysis. Figure 1 shows the chromatographic separation achieved using a 10 mM ammonium acetate/methanol gradient and heating the analytical column to 70 C. Previous internal standards used were characterized by poor day-to-day precision resulting in frequent calibration. PBA is currently being evaluated as a potential internal standard and BBA as a potential surrogate. The internal calibration using PBA is linear with r2>0.995 for all target analytes. Long-term stability of the calibration using PBA as the internal standard is currently being studied.EPA drinking water regulatory methods typically use preservatives to prevent microbial degradation (e.g., acid, copper(II) sulfate, DZU, trinitro) and to dechlorinate (e.g., sodium sulfite, trizma) the sample. Microbial degradation of the target analytes cannot be predicted in all types of matrices containing various types of microbiological contaminants, thus an anti-microbial agent is desirable. While chlorine may not adversely affect the stability of acetanilide degradates, it can interfere in the solid phase extraction, thus the residual chlorine should be removed. A number of preservative combinations were investigated based on research conducted by Winslow and colleagues [10]: copper(II) sulfate/trizma, DZU/trizma, trisnitro/trizma, hydrochloric acid (pH=2)/sodium sulfite. Table 1 lists the recoveries and relative standard deviations (RSDs) obtained with the various preservatives. The recovery and precision goals for EPA methods are typically 70-130% recovery with <30% RSDs. Only the hydrochloric acid (pH=2)/sodium sulfite combination met these recovery and precision goals with the exception of alachlor SAA and the surrogate. Extractions performed using only trizma (no anti-microbial) resulted in acceptable recoveries indicating that in 200 mL samples, the trizma is not the problem. In the case of copper(II) sulfate, the sulfate anion may be interfering with the adsorption of the target acids onto the carbon SPE sorbent. In the case of DZU, retention of the highly concentrated DZU on the carbon cartridges is probably exceeding the capacity of the cartridge, thereby preventing retention of the target analytes. The extracts produced using the trisnitro/trizma combination were yellow, indicating retention of the trisnitro on the carbon sorbent similar to DZU. Analysis of the trisnitro/trizma extracts overwhelmed the LC/MS and yielded poor target analyte recoveries (not shown), thus studies with trisnitro were discontinued. The acid/sodium sulfite combination demonstrates the most promise as potential preservatives for this method. Further studies are in progress to study the effect of water quality parameters, such as humic material and hardness, on the recovery of these analytes using this procedure. In addition, holding time studies will be performed to

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Talley, J.M., Shoemaker, J.A., Fout, G.S., and Dahling, D.R. Strain differentiation and determination of capsid proteins of coxsackievirus by MALDI-MS. Presented at: 224th American Chemical Society National Meeting, Boston, MA, August 18-22, 2002. 8/18/2002

Contact: Jody A. Shoemaker

Abstract: Introduction: Contamination of viruses in water environments (rivers, lakes, sources of drinking water) is a new threat and serious health problem. Amongst the organisms listed on the EPA 1998 Contaminant Candidate List (CCL) is the Coxsackievirus.1 The viruses are discharged from sewage and septic systems, are often adsorbed onto solid surfaces (suspended solids and sediments) and keep their activity for a long time.2-3 Coxsackieviruses are non-enveloped viruses that contain a single-stranded RNA genome. The virus genome serves directly as the source of information for protein synthesis. Coxsackie infection is more virulent in children than adults. Symptoms may include flu-like bodily aches accompanied by fever and diarrhea and may be followed by myocarditis and chest pain. The severity of the symptoms depends on the strain of virus, and on the age, gender and genetic background of the host.4 Differentiation between different strains of the Coxsackievirus using conventional biological methods takes up to several months. The capsid protein sequences of several Coxsackie strains are known and therefore amenable to rapid analysis by matrix-assisted laser desorption (MALDI) mass spectrometry (MS). Capsid proteins protect the virus genome and therefore give insight on infectivity. This study represents the first investigation of the four capsid proteins of Coxsackie A and B viruses by MALDI-MS. Materials and Methods: Virus Propagation: 250 ml flasks were inoculated with 25 ml of media and 1 ml of a strain of either Coxsackie A or B viruses (originally propagated from water samples) and incubated at 37 C for five to seven days. When the cytopathic effect (CPE) indicated that virus replication had occurred, the samples were frozen in a -70 C freezer until mass spectrometric analysis.Virus Purification: Virus purification consisted of underlaying the viral sample with sucrose and pelleting the virus through a sucrose pad in a Beckman SW 50.1 rotor at 45,000 rpm at 10 C for two hours. The virus was layered atop a 7.5%-45% sucrose gradient, centrifuged at 10 C for 1.5 hours and fractionated. The virus was then layered atop a 20-45% CsCl gradient and centrifuged at 10 C overnight. The virus was collected and desalted with a microcon-100 concentrate. Matrix Assisted Laser Desorption Ionization (MALDI) Mass Spectrometry (MS): The viral sample was reacted with 0.5% trifluroacetic acid (TFA) in water and mixed with a matrix consisting of sinapinic acid in 70/30 (v/v) acetonitrile / 0.1% TFA water. Acid-resistant lab label protection tape was applied to the target and acted as a hydrophobic surface. This prevented the spot from spreading and protected the target surface from the use of strong acids. MALDI-MS experiments were performed on a Bruker Biflex III. Thermal relaxation of the excited matrix molecules resulted in adsorption of the capsid protein into the gas phase. All capsid proteins were run in the linear mode and detected by the first detector by their mass-to-charge (m/z) ratio.Results and Discussion: Reacting the virus with 0.5% TFA water disrupted the protein capsid and released the four viral capsid proteins (VP1, VP2, VP3, and VP4). Preliminary results from the capsid protein of the Coxsackie B6 virus show four peaks corresponding to the four viral proteins (Figure 1), m/z 33,044 (VP1[H]+), m/z 13,599 (VP2[H]2+), m/z 13,047 (VP3[H]2+) and m/z 7,531 (VP4[H]+). Note that the singly charged species was not observed for VP2 and VP3, only the doubly charged species. The calculated weight for VP1, VP2 and VP3 was within 0.3-0.5% of the actual molecular weight. The low resolution and the low accuracy of MALDI in the linear mode can account for the molecular weight differences at the higher masses. However, the actual molecular weight of VP4 was 7,528 Dalton (Da.) which was only three Da. off from the experimental m/z. Coxsackie B5 showed similar results. On the other hand, Coxsackie A20 (Figure 2) only showed two VP peaks corresponding to VP1[H]+ at m/z 32,913 and VP4[H]+ at m/z 7,503. This was most likely due to a low viral titer since baseline resolution was not achieved causing VP2 and VP3 to be buried in the noise. The results thus far show that differentiation between strains of Coxsackievirus can be achieved by using VP4 as a biomarker. Coxsackie B3 is also under investigation at this time. The information we obtain will aid microbiologists in developing a more rapid method of identifying viruses in drinking water.

McDaniels, A.E., Covert, T.C., De La Cruz, AA, and Rodgers, M.R. Bacterial pathogenic research in response to contaminant candidate list needs. Presented at: EPA Science Forum 2002, Washington, DC, April 30 - May 2, 2002. 4/30/2002

Contact: Mark R. Rodgers

Abstract: The Safe Drinking Water Act, as amended in 1996, requires EPA to establish a Contaminant Candidate List (CCL) of unregulated microbiological and chemical contaminants to aid in priority setting for the Agency's drinking water program. At predetermined intervals the Agency must select five or more contaminants from the current CCL and determine whether they should be regulated. Information on where these contaminants occur and the extent of exposure in humans, with particular emphasis on the effects that these contaminants have on sensitive subpopulations, such as infants, children, pregnant women, the immunocompromised, and the elderly, will be needed to support any future regulatory decisions. There are four bacteria on the current CCL: Mycobacterium avium complex, Aeromonas, Helicobacter pylori and cyanobacterial toxins. Each of these is different with regard to the state of the art of the available detection methodologies and therefore our knowledge of their possible occurrence in distribution system drinking water. We know that Mycobacterium avium complex bacteria occur naturally in the environment and are relatively resistant to standard disinfection practices. A typical cultural assay for these organisms can take up to eight weeks to complete and the sensitivity of the assay is low due to the harsh decontamination steps necessary to prevent overgrowth of faster growing competing bacteria. We have developed a molecular DNA method which includes a one week enrichment step. We have demonstrated good agreement between this molecular method and the standard cultural assay. Aeromonas bacteria are also naturally occurring in all fresh waters and have been found in distribution system water, possibly due to their multiplication in biofilm material. Our evaluation, modification and validation studies have resulted in EPA Draft Method 1605. This method will be utilized in the monitoring requirements of the Unregulated contaminant Monitoring Rule. Our work with cyanobacteria, another commonly encountered group of bacteria in environmental waters, is focused on the toxins these organisms produce and we are developing sensitive methods based on the use of bio-sensors. The natural source(s) Helicobacter pylori, the causative agent of most gastric ulcers and the only recognized bacterial carcinogen, are unknown, as are the primary routes of its transmission in humans. We are developing and evaluating both cultural and molecular methods of detection for this important pathogen.

Stelma, Jr., G.N., Lye, D.J., Smith, B.G., and Messer, J.W. Rare occurrence of heterotrophic bacteria with pathogenic potential in potable water. Presented at: Joint Workshop-National Sanitation Foundaton/World Health Organization, Geneva, Switzerland, April 22-24, 2002. 4/22/2002

Contact: Gerard N. Stelma

Abstract: Since the discovery of Legionella pneumophila, an opportunistic pathogen that is indigenous to water, microbiologists have speculated that there may be other opportunistic pathogens among the numerous heterotrophic bacteria found in potable water. The USEPA developed a series of rapid in vitro assays to assess the virulence potential of large numbers of bacteria from potable water to possibly identify currently unknown pathogens. Results of surveys of potable water from several distribution systems using these tests showed that only 50 of approximately10,000 bacterial colonies expressed one or more virulence characteristics. In another study, 45 potable water isolates that were isolated from granular activated carbon filters and expressed multiple virulence factors were tested for pathogenicity in immunocompromised mice. None of the isolates infected mice that were compromised either by treatment with carrageenan, to induce susceptibility to facultative intracellular pathogens, or by cyclophosphamide, to induce susceptibility to extracellular pathogens. These results indicate that there are very few potential pathogens in potable water and that the currently developed in vitro virulence screening tests give an overestimation of the numbers of heterotrophic bacteria that may be pathogens. Current efforts are focused on using the animal models to screen concentrated samples of waters known to contain large numbers of heterotrophic bacteria and newly discovered Legionella-like organisms that parasitize amoebae.

Vesper, S.J., and Haugland, R.A. Rapid identification and quantification of fungi. Presented at: EPA Forum 2002, Washington, DC, May 1-2, 2002. 5/1/2002

Contact: Stephen J. Vesper

Abstract:

Vesper, S.J. A revolution in mold identification and enumeration. Presented at: Aerias Symposium on Mold, Atlanta, GA, October 17, 2002. 10/17/2002

Contact: Stephen J. Vesper

Abstract:

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JOURNAL

Grimm, A.C., and Fout, G.S. Development of a molecular method to identify hepatitis E virus in water. Journal of Virological Methods (Elsevier) 101 (2):175-188 (2002). www.elsevier.com/locate/jviromet. — 1/1/2002

Contact: Ann Grimm

Abstract: Hepatitis E virus (HEV) causes an infectious form of hepatitis associated with contaminated water. By analyzing the sequence of several HEV isolates, a reverse transciption-polymerase chain reaction method was developed and optimized that should be able to identify all of the known HEV strains. When tested under laboratory conditions, this method was able to detect low levels of five diverse HEV variants. In addition, internal controls were constructed so that any PCR inhibition could be detected. Finally, virus-spiked environmental water samples were successfully analyzed with these assays.

Morgan, J.N., Rosenblum, L., and Garris, S. Comparison of five extraction methods for determining incurred and fortified pesticides in dietary composites. Journal of AOAC International 85 (5):1167-1176 (2001). — 9/1/2002

Contact: Jeffrey N. Morgan

Abstract:

Gallagher, P.A., Creed, J.T., Schwegel, C.A., Murray, S., and Wei, X. An evaluation of sample dispersion medias used with accelerated solvent extraction for the extraction and recovery of arsenicals from LFB and DORM-2. Journal of Analytical Atomic Spectrometry (The Royal Society of Chemistry) 17 (6):581-586 (2002). www.rsc.org/jaas. — 5/27/2002

Contact: Patricia Gallagher

Abstract: An accelerated solvent extraction (ASE) device was evaluated as a semi-automated means for extracting arsenicals from quality control (QC) samples and DORM-2 [standard reference material (SRM)]. Unlike conventional extraction procedures, the ASE requires that the sample be dispersed in an inert dispersion media prior to the extraction. The need to disperse the sample in a support matrix prior to extraction is demonstrated by a 58% reduction in the extraction efficiency of arsenic (As Extraction Efficiency) from DORM-2 if the sample is not homogeneously suspended in the dispersion media.

Gamble, B.M., Gallagher, P.A., Shoemaker, J.A., Wei, X., Schwegel, C.A., and Creed, J.T. An investigation of the chemical stability of arsenosugars in simulated gastric juice and acidic environments using IC-ICP-MS and IC-ESI-MS/MS. Analyst 127 (6):781-785 (2002). www.rsc.org/analyst. — 4/23/2002

Contact: John T. Creed

Abstract: A more quantitative extraction of arsenic-containing compounds from seafood matrices is essential in developing better dietary exposure estimates. More quantitative extraction often implies a more chemically aggressive set of extraction conditions. However, these conditions may result in undesirable chemical changes in the native arsenical which may further complicate the toxicological risk assessment. This balance between quantitative extraction and species-specific integrity may be best addressed by using simulated gastric juice as an extraction solvent to mimic 'bioavailability'. This, conceptually, should extract the bioavailable fraction and induce any chemical changes that would occur because of ingestion. The most chemically labile species associated with seafood are thought to be the arsenosugars and for this reason their chemical stability is investigated in this study. Four arsenosugars [As(328); As(392); As(408); and As(482)] were isolated from seaweed extracts and subjected to simulated gastric juice and acidic conditions which mimic the stomach's pH of 1.1. Three acid solutions were used to test the chemical stability of the arsenosugars: simulated gastric juice, 78 mM nitric acid and 78 mM hydrochloric acid. The composition of the solutions was monitored over time (up to 48 h) using IC-ICP-MS for detection. The arsenosugars were found to degrade at the rate of 1.4% per h at 38 degrees c and 12.2% per h at 60 degrees C. The plots of percent conversion versus time were found to be independent of the starting arsenosugar and all had r 2 values of greater than 0.97. A single common degradation product was observed in all the stability studies. A mass balance between the starting arsenosugar [As(392), As(408) and As(482)] and the degradation product was conducted with each set of experiments. This mass balance indicated that the degradation process did not produce any unchromatographable species. This degradation product was tentatively identified as As(254) as determined by ESI-MS/MS spectral data. An acid hydrolysis mechanism was proposed for the formation of As(254) from each of the native arsenosugars by hydrolysis at the C-1 carbon on the ribose ring.

Wymer, L.J., and Dufour, A.P. A model for estimating the incidence of swimming-related gastrointestinal illness as a function of water quality indicators. Environmetrics (John Wiley & Sons, Inc.) 13 (5-6):669-678 (2002). 9/30/2002

Contact: Larry J. Wymer

Abstract: Several studies have demonstrated association between illnesses, in particular gastrointestinal illness (GI), in swimmers and sewage pollution as measured by the density of indicator organisms, such as E. coli and enterococci, in recreational waters. These studies generally classify illnesses into two categories according to the subjectivity of the reported symptoms and utilize separate analyses on the incidence of total illness and the incidence of objective symptoms of gastroenteritis. Generally, non-swimmer illness rates are available from these studies as an indicator of the background illness rates, but are not always utilized in the model. Data from two prospective epidemiological studies conducted by the EPA and evidencing relationships between the incidence of swimming-associated GI and enterococcus or E. coli density in marine and fresh water, respectively, are used as examples. Initially, analysis of these data consisted of linear regression of log10 enterococcus density on the difference in illness rates between swimmers and non-swimmers. Subsequent published analysis of the marine study utilized logistic regression, but did not take background illness rates into account. Both analyses produced separate models for rates of "highly credible" and total GI symptoms. The present analysis demonstrates the advangages of including the background rates and how such rates may be incorporated in a logistic regression.

Roe, J.D., Haugland, R.A., Vesper, S.J., and Wymer, L.J. Quantification of stachybotrys chartarum conidia in indoor dust using real time, fluorescent probe-based detection of PCR products. Journal of Exposure Analysis and Environmental Epidemiology (Nature Publishing Group) 11:1-9 (2001). www.nature.com/jea. 2/27/2002

Contact: Richard A. Haugland

Abstract: Analyses of fungal spores or conidia in indoor dust samples can be useful for determining the contamination status of building interiors and in signaling instances where potentially harmful exposures of building occupants to these organisms may exist. A recently developed method for the quantification of stachybotrys chartarum conidia, using real time, fluorescent probe-based detection of PCR products (TaqMan system) was employed to analyze indoor dust samples for this toxigenic fungal species. Dust samples of up to 10 mg were found to be amenable to DNA extraction and analysis. Quantitative estimates of S. chartarum conidia in composite dust samples, containing a four log range of these cells, were within 25-104% of the expected quantities in 95% of analyses performed by the method. Calibrator samples containing known numbers of S. chartarum conidia were used as standards for quantification. Conidia of an arbitrarily selected strain of Geotrichum candiidum were added in equal numbers to both dust and calibrator samples prior to DNA extraction. Partial corrections for reductions in overall DNA yields from the dust samples compared to the calibrator samples were obtained by comparative analyses of rDNA sequence yields from these reference conidia in the two types of samples. Dust samples from two contaminated homes were determined to contain greater than 10-3 S. chartarum conidia/mg in collection areas near the sites of contamination and greater than 10-2 conidia/mg in several areas removed from these sites in analyses performed by the method. These measurements were within the predicted range of agreement with results obtained by direct microscopic enumeration of presumptive Stachybotrys conidia in the same samples.

Vesper, S.J., and Vesper, M. J. Stachylysin may be cause of hemorrahaging in stachybotrys chartarum exposures. Infection and Immunity (American Society for Microbiology) 70 (4):2065-2069 (2002). 4/1/2002

Contact: Stephen J. Vesper

Abstract: Stachybotrys chartarum is a toxigenic fungus that has been associated with human health concerns like nasal bleeding in adults and pulmonary hemosiderosis (PH) in infants. Stachylysin is a glycosylated protein, with the deglycosylated molecular mass of 21.5 kDa. Seven of eight strains of S. chartarum isolated from homes of PH infants in Cleveland and the strain from the lung of a PH infant in Texas produced stachylysin in tryptic soy broth (TSB) whereas only one out of eight strains isolated from control homes produced stachylysin. However, all strains produced stachylysin when grown on TSB with 0.7 % sheep's blood. When stachylysin was injected into Lumbricus terrestris, the erythrocruorin hemoglobin (absorbance peaks at 280 and 415 nm) was released resulting in a lethal effect. These results support the hypothesis that stachylysin may be responsible for hemorrhaging in humans.

Haugland, R.A., Brinkman, N., and Vesper, S.J. Evaluation of rapid DNA extraction procedures for the quantitative detection of fungal cells using real time PCR analysis. Journal of Microbiological Methods (Elsevier Science B.V.) 50 (3):319-323 (2002). www.elsevier.com/locate/jmicmeth. 8/1/2002

Contact: Richard A. Haugland

Abstract: The ease and rapidity of quantitative DNA sequence detection by real-time PCR instruments promises to make their use increasingly common for the microbial analysis many different types of environmental samples. To fully exploit the capabilities of these instruments, correspondingly rapid, easy and reproducible methods for extracting microbial DNA from these samples need to be identified or developed. To address this need, we evaluated three new methods for the extraction of DNA from fungal cells that offer significant savings in time and effort over our previously reported procedures. Existing or newly developed TaqMan PCR assays for four fungal species: Aspergillus fumigatus, Stachybotrys chartarum, Candida albicans and Geotrichum candidum; and an established comparative threshold quantitative analysis method were used to determine the extraction methods' yields of target DNA from known cells in the presence of different, commercially available lysis reagents gave similar DNA yields to those of our most efficient previous method while the third, which did not use bead milling gave significantly lower yields. Both of the bead milling methods were found to support accurate and precise quantification of target fugal cells in air and water samples, however only one of these methods, involving further purification of the DNA by a streamlined silica adsorption procedure was found to be adequate for the analysis of building dust samples.

Viana, M.E., Selgrade, M.K., Vesper, S.J., and Ward, M.D.W. An extract of Stachybotrys chartarum causes allergic responses in a BALB/c mouse model: I. Biochemical and pathological responses. Toxicological Sciences (Oxford University Press) 70:2065-2070 (2002). 12/1/2002

Contact: Stephen J. Vesper

Abstract: Environmental exposure to Stachybotrys chartarum has been associated with adverse health effects in humans. The goal of this study was to assess soluble components of this fungus for allergenic potential. Five isolates of S. chartarum were combined and extracted to form a crude antigen preparation (SCE-1). Female BALB/c mice were sensitized by involuntary aspiration of SCE-1 and subseqently re-exposed at 2, 3, and 4 weeks. To distinguish immune from non-specific inflammatory effects, mice were exposed to 3 doses of Hank's Balanced Salt Solution (HBSS) and a final dose of SCE-1; or to 4 doses of BSA as a negative control protein. Serum and bronchoalveolar lavage fluid (BALF) were collected before the fourth aspiration (day 0), and at days 1, 3, and 7 following the final exposure, and lungs were fixed for histopathological examination. SCE-1-exposed mice displayed increased BALF total protein on days 0, 1, and 3, and increased lactate dehydrogenase (LDH) at days 1 and 3 compared to HBSS controls. BALF total cell numbers were elevated on each day, and differential counts of BALF cells showed neutrophilia on day 1, marked eosinophilia on all days, and increased numbers of lymphocytes at days 1, 3, and 7. Serum and BLAF total IgE levels were elevated at all days, and BLAF IL-5 levels were greatly increased (7-fold) on day 1. Mice exposed to a single dose of SCE-1 exhibited inflammatory responses but not allergic responses, while BSA-treated mice showed neither inflammatory nor allergic responses. Histopathology confirmed the biochemical findings. The data indicate a transient non-specific inflammatory response followed a single or multiple exposure to SCE-1. Following multiple exposures, there was a more sustained immuneomediated eosinophilia and IgE response. We conclude that multiple respiratory exposures to SCE-1 cause responses typical of allergic airway disease in this mouse model, and that BSA can serve as a negative control protein when administered by this route.

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Lindquist, H.D.A. Emerging waterborne pathogens. Presented at: 52nd Annual KU Environmental Engineering Confernece, Lawrence, KS, February 6, 2002. 2/6/2002

Contact: H. d. alan Lindquist

Abstract: In 1971, the U.S. Environmental Protection Agency (EPA) entered into a long term agreement with the Centers for Disease Control and Prevention (CDC) to gather data on the occurrence of waterborne illness. It is difficult however to know what effects increases in surveillance have had on the perceived incidence of disease. For example, in Figure 1, the number of outbreaks of waterborne disease increased sharply in the 1970's, which coincides with the undertaking of this surveillance program. The incidence of disease follows a similar pattern (Figure 2) with the exception of the tremendous spike cases in the 1990's due to the Milwaukee outbreak. (Moore AC, 1993, Kramer, et al. 1996, Levy, et al. 1998, and Barwick, et al. 2000). Contaminated drinking water is no longer a significant cause of death in the United States. Modern drinking water treatment systems have reduced or eliminated the large outbreaks of cholera and typhoid fever, that were major causes of mortality in the early settlement of the United States. These treatments are effective against a variety of diseases beyond the classical agents of drinking water mortality. However, waterborne diseases still cause a large number of cases of illness, and several deaths each year. Because of the widespread use of drinking water, there is potential for disastrously large outbreaks of disease if the water becomes contaminated with a suitable disease agent. As new disease agents are uncovered, treatment processes are optimized to control these agents. The appearance of new agents of waterborne disease is considered to be disease emergence.

Willian-True, S., Parshionikar, S., Newport, C., Robbins, D.E, and Fout, G.S. Detection of outbreak-associated human caliciviruses in groundwater by RT-PCR. Presented at: American Society for Virology Annual Meeting, Lexington, KY, July 20-24, 2002. 7/22/2002

Contact: G. shay Fout

Abstract: Human caliciviruses (HuCV) are a major worldwide cause of food and waterborne outbreaks of acute nonbacterial gastroenteritis, and have been placed on the U.S. Environmental Protection Agency's (U.S. EPA) Contaminant Candidate List (CCL) of agents to be considered for regulatory action. This activity has led to the development of a method to measure HuCV occurrence in drinking water. Viruses are considered the likely agent in over half of the waterborne outbreaks, but they have rarely been detected. HuCV are non-culturable and can only be detected in concentrated water samples using RT-PCR. In 2001, the U.S.EPA participated in the investigation of two HuCV outbreaks in the state of Wyoming. The first outbreak was recorded at a wintertime vacation lodge in northern Wyoming and the second during the summer at a restaurant in central Wyoming. For each outbrak a well water sample was concentrated for virus using a 1MDS positively charged cartridge filter. Filters were eluted with beef extract and the eluates concentrated with celite. The concentrated eluates were treated to remove PCR inihibitors and then assayed by RT-PCR using several HuCV-specific primer sets. HuCV were detected in the concentrated water samples from both outbreaks. Sequencing of the cloned RT-PCR products demonstrated the presence of a genogroup II, subtype 1 HuCV in the first outbreak and a genogroup II, subtype 3 HuCV in the second outbreak. These results demonstrate the utility of the RT-PCR method for investigations of waterborne outbreaks of suspected viral origin.

Piche, R., Chen, R., Hoang, L., Dixon, B., Cross, J.H., Lindquist, H.D.A., Fyfe, M.W., Champange, S., Isaac-Renton, J.L., and Ong, C.S.L. Molecular epidemiological studies on two cyclosporiasis outbreaks in Vancouver, British Columbia. Presented at: Emerging Waterborne Disease Symposium at the International Congress of Parasitology, Vancouver, BC, August 4-9, 2002. 8/4/2002

Contact: H. d. alan Lindquist

Abstract: Two cyclosporiasis outbreaks in Vancouver, British Columbia (BC) were investigated using molegular epidemiology. The cause of the 1999 outbreak has not been identiifed whereas the 2001 oubreak has been linked epidemiologically to the consumption of Thai basil. The internal transcribed spacer 1 (ITS1) region was used as the molecular marker. Sequencing of amplified 18S rRNA and ITS1 loci of isolates collected during the 1999 and 2001 outbreaks showed almost complete homology for all 18S rDNA sequences. A higher degree of variation in the ITS1 sequences was observed between isolates collected during different outbreak periods.

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Schaefer, III, F.W. Utility of surrogates for measuring Cryptosporidium oocyst infectivity. Presented at: Drinking Water Inspectorate Research Conference on Drinking Water 2002, Tradey Court, England, June 10-11, 2002. 6/10/2002

Contact: Frank W. Schaefer

Abstract: The water industry must assess whether Cryptosporidium oocysts detected in source and finished water areviable and/or infectious. Initial approaches measuring the infectious nature of C. parvum oocysts have focused on in vitro excystation and in vitro vital dye staining. Recently there has been increased interest in animal models and a Cryptosporidium cell culture assay. Studies comparing in vitro excystation and in vitro vital dye staining to the neonatal mouse model consistently showed both in vitro assays to over estimate the infectious nature of oocysts exposed to disinfectants. While human volunteer studies have been conducted to determine the infectious dose of various Cryptosporidium isolates, human volunteers have never been used for disinfection studies on these pathogens. Besides being extremely expensive, it is difficult to get human subjects approval to do such infectivity studies. Animal models have been used with some success, but they are only able to evaluate C. parvum genotype 2 isolated from bovids and humans and not C. parvum genotype 1 isolated only from humans. In addition, results from both human volunteers and animal models are extremely variable, as they are dependent on the strain and age of the host, when the oocysts were shed, the strain and particular isolate of C. parvu, the mehtods applied to enumerate the oosysts, the procedures used to inocuclate the oosysts, and the inter- and intra-strain variation in animal susceptibility to infection. Limited side by side studies comparing the cryptosporidium cell culture assay with the animal model have shown the two to produce comparable results in the case of C. parvum genotype 2 oocysts. Unlike the animal model, the Cryptosporidium cell culture assay is able to estimate infectivity of both C. parvum genotype 1 and genotype 2 oocysts.Of the approaches used to determine the infectivity of Cryptosporidum oocysts, the Cryptosporidum cell culture assay currently appears to be the most promising. However, at present there are a number of knowledge gaps associated with the Cryptosporidium cell culture assay which must be overcome before it can be used on a reliable, routine basis. The assay is not standardized as to the optimal cell line and culture medium formulation to be used. The sensitivity and specificity of the assay have yet to be determined in an impartial manner. Since a number of Cryptosporidum spp. including C. felis, C. canis, C. meleagridis, C. parvum genotype 1 and C. parvum genotype 2 now are known to infect humans the question is, will Cryptosporidium cell culture assay work equally well for each of these different species? Once a standardized assay is available, then a laboratory round robin validation must be conducted to determine how robust the assay is. Incorporation of the Cryptosporidium cell culture assay into a complete method which would allow concentrating and purifying oocysts from various environmental water matrices.

Ware, M.W., Wymer, L., Lindquist, H.D.A., and Schaefer, III, F.W. Evaluation of Cryptosporidium oocyst recovery in water by EPA method 1623 with a modified IMS dissociation procedure. Presented at: International Symposium on Waterborne Pathogens, Lisbon, Portugal, September 22-25, 2002. 9/22/2002

Contact: H. d. alan Lindquist

Abstract: U.S.EPA Methods 1622 and 1623 are used for the detection of waterborne Cryptosporium. These methods consist of filtration, elution, purificaiton by immunomagnetic separation (IMS), and microscopic analysis for oocysts stained by a fluorescent monoclonal antibody and counter stained with 4',6-diamidino-2-phenyl indole (DAPI). DAPI counter staining is used to confirm oocysts and reduce the number of false positives. This study compared the IMS acid dissociation with heat with heat only dissociation of the parasite from the immunomagnetic beads. The effect of this change was evaluated by analyzing 48 reagent and river water samples. The average oocyst recovery in these samples improved from 28% with acid dissociation to 63% with heat only dissociation. The average DAPI confirmation rate improved from 48% to 85%.

Schaefer, F.W. Laboratory guidelines for analysis of bioterrorism samples. Presented at: International Symposium of Waterborne Pathogens, Cascais/Lisbon, Portugal, September 22-25, 2002. 9/22/2002

Contact: Frank W. Schaefer

Abstract: With advent of deaths associated with Bacillus anthracis spore contaminated mail, a worldwide need was apparent for increased laboratory capacity to safely analyze bioterrorism samples. The U.S. Department of Health and Human Services has furnished guidelines for microbiological and biomedical laboratory safety. These guidelines are categorized into primary barriers dealing with safety equipment and secondary barriers dealing with facility. Other areas not readily apparent include monitoring the analyst's health, vermin and insect control, as well as government control of select agents and specialized reagents.

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Presented Published

Stelma, Jr., G.N., Lye, D.J., Smith, B.G., and Messer, J.W. Rare occurrence of heterotrophic bacteria with pathogenic potential in potable water. Presented at: NSF International/WHO Symposium on HPC Bacteria in Drinking Water, Geneva, Switzerland, April 22-24, 2002. 4/22/2002

Contact: Gerard N. Stelma

Abstract: Since the discovery of Legionella pneumophila, an opportunistic pathogen that is indigenous to water, microbiologists have speculated that there may be other opportunistic pathogens among the numerous heterotrophic bacteria found in potable water. The USEPA developed a series of rapid in vitro assays to assess the virulence potential of large numbers of bacteria from potable water to possibly identify currently unknown pathogens. Results of surveys of potable water from several distribution systems using these tests showed that only 50 of the approximately 10,000 bacterial colonies expressed one or more virulence characteristics. In another study, 45 potable water isolates that were isolated from granular activated carbon filters and expressed multiple virulence factors were tested for pathogenicity in immunocompromised mice. None of the isolates infected mice that were compromised either by treatment with carrageenan, to induce susceptibility to facultative intracellular pathogens, or by cyclophosphamide, to induce susceptibility to extracellular pathogens. These results indicate that there are very few potential pathogens in potable water and that the currently developed in vitro virulence screening tests give an overestimation of the numbers of heterotrophic bacteria that may be pathogens. Current efforts are focused on using the animal models to screen concentrated samples of waters known to contain large numbers of heterotrophic bacteria and newly discovered Legionella-like organisms that parasitize amoebae.

Kahane, D., McGinnis, M., Vesper, S.J., and Haugland, R.A. Comparison of genetic methods to optical methods in the identification and assessment of mold in the built environment -- comparison of TAQMAN and microscopic analysis of cladosporium spores retrieved from Zefon Air-O-Cell traces. Presented at: Indoor Air 2002, The 9th International Conference on Indoor Air Quality and Climate, Monterey, CA, June 30-July 5, 2002. 6/30/2002

Contact: Stephen J. Vesper

Abstract: RECENT ADVANCES IN THE SEQUENCING OF RELEVANT WATER INTRUSION FUNGI BY THE EPA, COMBINED WITH THE DEVELOPMENT OF PROBES AND PRIMERS HAVE ALLOWED FOR THE UNEQUIVOCAL QUANTITATIVE AND QUALITATIVE IDENTIFICATION OF FUNGI IN SELECTED MATRICES. IN THIS PILOT STUDY, QUANTITATIVE PCR WAS PERFORMED ON PREVIOUSLY ANALYZED ZEFON? CASSETTES TO VALIDATE METHODS OF EXTRACTION OF DEPOSITED SPORES, AND TO COMPARE RELATIVE QUANTITATIVE AGREEMENT Between the two methods of analysis. ZEFON? Cassettes are extensively used in airborne assessments for fungi, but are often supplemented by culture techniques to obtain species identification.The pilot study employed the comparison of Cladisporium obtained from field generated ZEFON? Samples. Thirty-two samples ranging in concentatoin from spores per cubic meter were analyzed and compared for general agreement.

Li, D., Haughland, R.A., Vesper, S.J., and Yang, C.S. Is your Stachybotrys chartarum a genuine S. chartarum?. Presented at: Indoor Air 2002, The 9th International Conference on Indoor Air Quality and Climate, Monterey, CA, June 30-July 5, 2002. 6/30/2002

Contact: Richard A. Haugland

Abstract: The fungus Stachybotrys chartarum is the type species of the genus Stachybotrys. Certain strains of the species are known to produce trichothecene mycotoxins,. It is a celluolytic saprophyte with worldwide distribution and frequently discovered in water-damaged buildings. Evidences of the detrimental effects on human health due to respiratory exposure to this fungus have been reported. However, morphological and mycotoxin profile studies showed that this species is not well delineated. This presentation reviews and summarizes data and evidences regarding the taxonomic status of the epithet S. chartarum.